MICROBIOTEST
A Division of Microbac Laboratories, Inc.
105-B Carpenter Drive
Sterling, VA 20164

MICROBIOTEST PROTOCOL

AOAC USE DILUTION FUNGICIDAL TEST
Using *Microsporum canis*

Testing Facility
MICROBIOTEST
A Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Virox Technologies, Inc.
2770 Coventry Road
Oakville, Ontario
L6H 6R1

March 29, 2012

OBJECTIVE:

This test is designed to determine disinfectant effectiveness for a product to be used as a disinfectant. It measures the potential of the test agent to disinfect hard surfaces contaminated with fungus. The test is based on the one in *Official Methods of Analysis* (2006/2009) and EPA 810.2200.

TESTING CONDITIONS:

Using a single test agent, a total of 10 replicates per lot will be evaluated using two lots of the test agent. *Microsporum canis* cultures dried on stainless steel penicylinders will be exposed to each lot of the test agent at the temperature and for the time stipulated by the sponsor. The carriers will be removed from the test agent, neutralized and cultured.

MATERIALS:

A. Test agent supplied by the sponsor: see last page.

The test agent will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test agent such as dilution or specialized storage conditions must by specified by the sponsor prior to the initiation of testing.

The sponsor assures MICROBIOTEST, a Division of Microbac Laboratories, Inc. (MICROBIOTEST) testing facility management that the test agent has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MICROBIOTEST will retain all unused test agents for a period of at least three months after completion of the test, and then discard them in a manner that meets the approval of the safety officer.

B. Materials supplied by MICROBIOTEST, including, but not limited to:

1. Challenge microorganism required by the sponsor of the study: *Microsporum canis*, ATCC 10214

2. Media and reagents:
   a. Emmon's Modification of Sabouraud's Agar (EMA)
   b. Deionized water
   c. Sodium hydroxide solution, 1N (NaOH)
   d. Recovery broth with neutralizers: Sabouraud Dextrose Broth containing 0.2% $Na_2S_2O_3$ and 0.1% Catalase
   e. Phosphate Buffered Saline (PBS)
   f. Sterile saline (SS)
   g. Heat-inactivated horse serum (if required)

3. Laboratory equipment and supplies including polished stainless steel penicylinders

TEST SYSTEM IDENTIFICATION:

All test and control tube racks will be labeled with microorganism, test agent (if applicable) and project number prior to initiation of the study and during incubation. Petri dishes will be labeled with microorganism prior to initiation of the study and microorganism and project number during incubation.

EXPERIMENTAL DESIGN:

A. Inocula preparation:

The fungus will be inoculated from the stock culture onto EMA plates and incubated at 25-30C for no less than ten, but no more than 15 days or until sporulation occurs. When the cultures appear to be mature, the mycelial mats will be removed from the surface of at least five plates and macerated with SS in a sterile glass tissue grinder. The suspension will be filtered through sterile glass wool to remove the hyphae.

The density of the conidial suspension will be determined by serially diluting the prepared culture in SS. Aliquots from selected dilutions will be plated on duplicate EMA plates. The plates will be incubated for 3-5 days at 25-30C. The suspension will be stored at 2-10C for less than four weeks before use. On the day of the test, the suspension will be adjusted to yield at least 5.0×10^6 conidia forming units (CFU) per mL, by dilution with SS. If requested by the sponsor, serum will be added to the culture to achieve a 5% organic load.

B. Carrier preparation:

The carriers will be soaked overnight in 1N NaOH, rinsed with tap water until a neutral pH is reached, then rinsed twice with deionized water. Cleaned carriers will be placed in multiples of 10 into sterile tubes, covered with deionized water, steam-sterilized for 20 minutes at 121C, cooled and stored at room temperature until use. The carriers will be placed into the broth and remain in contact with the inocula (20 carriers per tube of 20 mL inocula) for 15±2 minutes at ambient temperature; then they will be removed from the broth and placed into sterile, Petri dishes matted with filter paper, and dried at 36±1C for 40±12minutes.

C. Test agent preparation:

The test agent will be prepared according to the sponsor's specifications and dispensed in 10 mL aliquots into sterile test tubes. The tubes will be allowed to come to testing temperature for at least ten minutes before testing.

D. Test:

Tubes containing the test agent will be maintained at the testing temperature throughout the test. One contaminated carrier will be added to each tube; the tube swirled to mix; and the carrier allowed remaining in contact with the test agent for the times specified by the sponsor of the study. After the contact time, the carriers will be removed, transferred to recovery broth with neutralizers and the tubes will be thoroughly shaken. All tubes will be incubated at 25-30C for up to ten days and the results recorded as visible growth or no visible growth.

E. Controls:

1. Sterility controls:

One tube of recovery broth with neutralizers containing a single sterile carrier will be incubated with the test.

2. Neutralizer effectiveness:

One tube containing ten mL of the test agent, per lot, will be allowed to equilibrate to testing temperature for at least 10 minutes. A single sterile carrier will be added to the tube and held for the exposure time used for the test carriers. After the contact time, the carrier will be added to a tube containing recovery broth with neutralizers. Fewer than 100 colony forming units (CFU) of the challenge microorganisms will be added to the tube. The CFU added to each tube will be confirmed in duplicate EMA plates. The tubes will be incubated with the test. The plates will be incubated for 3-5 days at 25-30C.

3. Carrier counts:

The average CFU per carrier will be determined using three inoculated carriers. Dried carriers will be placed individually into tubes containing 10 mL recovery broth with neutralizers. The tubes will be subjected to ultrasound in a cleaning sonicator. Serial ten-fold dilutions of each suspension will be performed in PBS blanks. Duplicate 0.1 mL aliquots from selected dilutions will be plated in EMA plates. All plates will be incubated for 3-5 days at 25-30C.and the average CFU/carrier determined.

4. Viability controls:

Two inoculated carriers will be inoculated into tubes of recovery broth with neutralizers and incubated with the test to serve as comparison for the test cultures.

5. Inoculum counts:

   Serial ten-fold dilutions of the suspension will be performed in PBS blanks. Duplicate 0.01 mL aliquots from selected dilutions will be plated in EMA plates. All plates will be incubated 3-5 days at 25-30C and the average CFU/mL determined.

6. Fungistasis control:

   If, after ten days incubation, no growth is observed in any of the test tubes, at least 20% of the test tubes will be streaked onto EMA and incubated for 3-5 days at 25-30C. No growth on these plates will negate fungistasis as the cause for lack of growth in the test tubes.

7. Confirmation of challenge microorganisms:

   Following incubation of the test and control tubes, all of test tubes demonstrating turbidity and all viability tubes will be streaked onto EMA plates and incubated for 3-5 days at 25-30C. Wet mounts will be performed on all viability tubes and all of the colonies that appear to be the challenge microorganism from the test streaks. Preparations will be compared to that of a viability control tube.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the criteria listed below are satisfied. The study director may consider other causes that may affect test reliability and acceptance.

- The carrier counts should be greater than 1×10^4 - 1×10^5 CFU/carrier per EPA 810.2200.
- The neutralizer should be effective and support growth of the challenge microorganism(s).
- The inoculum counts should be at least 1×10^6 CFU/mL.

PRODUCT EVALUATION CRITERIA:

The compound passes the test if no visible growth is observed in any of the subculture broth tubes (0/10) per lot per microorganism and the controls meet their stipulated criteria. There is no statistical method proposed for this protocol.

DATA PRESENTATION:

The final report will include the following information:

- The number of positive carriers.
- The average colony-forming units per carrier.
- The results of all controls.

CONFIDENTIALITY:

All data generated at MICROBIOTEST are held in strictest confidence and are available only to the sponsor. In turn, no reference to the work, data, or MICROBIOTEST may be made public without the written consent of MICROBIOTEST.

REPORT FORMAT:

MICROBIOTEST employs a standard report format for each test design. Each final report provides the following information:

- Sponsor identification and test agent identification
- Type of test and project number
- Dates of study initiation and completion
- Interpretation of results and conclusions
- Test results
- Methods and evaluation criteria
- Signed Quality Assurance and Compliance Statements (if applicable)

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge microorganism used; media and reagent identification; and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets will be forwarded to the study sponsor.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164.

MISCELLANEOUS INFORMATION:
The following information is to be completed by sponsor before initiation of study:

A. Name and address: Virox Technologies, Inc.
2770 Coventry Road
Oakville, Ontario
L6H 6R1

B. Test agent: Accel Concentrate US

Active ingredient(s): H_2O_2

Lot No.'s: 1: 9342 2: 9343

Dilution to be tested: ☒ 1:16 (1 part test agent + 16 parts diluent)
☐ Ready to use (RTU)

Diluent: 200 AOAC Hard Water (±2.9%)
☐ Other: _____
☐ Not applicable (RTU)

Contact time: 5 min (must be ≤ 10 minutes)
Exposure temperature: Ambient room temperature 20±1C

Organic load – serum added to achieve 5% in the inoculum: ☒ yes ☐ no

C. Precautions/storage conditions: refer to MSDS or C of A: ☒ yes ☐ no

D. Additional information: _____

REPORT HANDLING: The sponsor intends to submit this information to: ☒ US EPA
☐ US FDA ☐ Health Canada ☐ CAL DPR ☐ ARTG ☐ other: Internal Purposes

STUDY CONDUCT: ☒ GLP ☐ non-GLP

PROTOCOL APPROVAL:

Sponsor Signature: _____ Date: 03/29/2012

Printed Name: NAVID OMIDBAKHSH

Study Director Signature: _____ Date: 04/12/12

Printed Name: SHIRSHENDU SAHA